92.5 milliliters of 37 percent hydrochloric acid.

(c) *Preparation of spotting solutions*— (1) *Sample solution.* Prepare a solution of the sample in anhydrous methanol to contain 10 milligrams per milliliter.

NOTE: It is advisable to prepare the sample and standard solutions immediately before spotting to minimize the possibility of degradation in solution.)

(2) *Standard solution.* Prepare a solution of erythromycin base reference standard in anhydrous methanol to contain 1 milligram per milliliter. Weigh 99.5, 99.0, and 97.0 milligrams of erythromycin estolate (propionyl erythromycin lauryl sulfate) reference standard and transfer to separate 10-milliliter volumetric flasks. To these flasks add 0.5, 1.0, and 3.0 milliliters, respectively, of the 1-milligram-per-milliliter solution of erythromycin base reference standard and dilute to volume with anhydrous methanol. These solutions contain, respectively, 0.5 percent, 1.0 percent, and 3.0 percent erythromycin base in erythromycin estolate. Prepare a solution of erythromycin estolate reference standard in anhydrous methanol to contain 10 milligrams per milliliter. Prepare a solution of erythromycin base reference standard in anhydrous methanol to contain 0.1 milligram per milliliter.

(d) *Procedure.* Pour 100 milliliters of developing solvent into the glass trough on the bottom of the unlined chromatography tank. Cover and seal the tank. Allow it to equilibrate while the plate is being prepared. Prepare a plate as follows: On a line 2.0 centimeters from the base of the thin-layer plate, apply 1.0 microliter of each of the following solutions:

(1) 10-milligrams-per-milliliter solution of erythromycin estolate reference standard, equivalent to 10 micrograms of erythromycin estolate;

(2) 0.5 percent base-in-estolate solution, equivalent to 0.05 microgram of base and 9.95 micrograms of estolate;

(3) 1.0 percent base-in-estolate solution, equivalent to 0.10 microgram of base and 9.90 micrograms of estolate;

(4) 3.0 percent base-in-estolate solution, equivalent to 0.30 microgram of base and 9.70 micrograms of estolate;

(5) 0.1-milligram-per-milliliter solution of erythromycin base reference standard, equivalent to 0.1 microgram of erythromycin base; and

(6) Sample solution, equivalent to 10 micrograms of erythromycin estolate. Allow the spots to dry. Place the plate directly in the chromatograph tank. Cover and seal the tank. Allow the solvent front to travel a distance of 7 centimeters (about 27 minutes). Remove the plate from the tank, and allow it to air dry under a hood. With the plate still under the hood, spray uniformly with the spray solution. Heat the sprayed plate in an oven at 100 °C for 5 minutes. (CAUTION: Avoid exposure to the acid fumes while removing the plate from the oven.)

(e) *Evaluation.* Erythromycin base and erythromycin estolate appear as reddish-violet spots on the sprayed and heated plate. Better visualization of the erythromycin base spots may be gained by viewing the plate under long-wavelength (366 nanometers) ultraviolet light, erythromycin base appearing as dark spots on a yellow-green fluorescent background. Erythromycin base has an R_f value of about 0.3. Erythromycin estolate has an R_f value of about 0.7. Compare the size and intensity of any erythromycin base spots in the sample lane with the erythromycin base spots in the erythromycin base reference standard lane and in the 0.5 percent, 1.0 percent, and 3.0 percent base-in-estolate lanes, and report the percentage of erythromycin base (free erythromycin) in the sample. For a more accurate determination of free erythromycin content, it may be necessary to repeat the test using a different set of standards.

[53 FR 1919, Jan. 25, 1988]

§ 436.363 High-performance liquid chromatographic assay for cefmenoxime.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 18-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material

as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 30 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the apparatus described in paragraph (a) of this section; and the reagents and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by 5 replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Using the apparatus and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and

f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left[\frac{t_R}{W_h}\right]^2$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of solute; and

w_h=Peak width at half-height.

(3) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2\left(t_{RJ} - t_{Ri}\right)}{w_i + w_J}$$

where:

t_{RJ}=Retention time of a solute eluting after i (t_{RJ} is larger than t_{Ri});

t_{Ri}=Retention time of any solute;

w_i=Width of peak at baseline of any solute; and

w_J=Width of peak at baseline of any solute eluting after i.

(4) *Coefficient of variation (Relative standard deviation).*

Calculate the coefficient of variation (S_R) in percent) as follows:

$$\underline{S_R} = \frac{100}{\overline{\underline{X}}}\left[\frac{\sum_{i=1}^{N}(\underline{X_i} - \overline{\underline{X}})^2}{\underline{N} - 1}\right]^{\frac{1}{2}}$$

where:

X is the mean of N individual measurements of X_i. If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[53 FR 13401, Apr. 25, 1988; 53 FR 19368, May 27, 1988]

§ 436.364 Atomic absorption test for sodium carbonate content of cefmenoxime hydrochloride for injection.

(a) *Apparatus.* A suitable atomic absorbance spectrophotometer equipped with:

(1) A suitable sodium hollow-cathode discharge lamp;

(2) An oxidizing air-acetylene flame;

(3) A nebulizer-burner system;